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METABOLIC RESPONSE TO STRESS -

TOBACCO SMOKE INTERACTIONS

Summary of Progress

For approximately 2½ years our laboratory has been studying stress-tobacco smoke interactions at a molecular metabolic level. Experiments were concerned with either acute (electroshock, immobilization) or chronic (isolation housing) stress. In the mouse, these have been quantified for their behavioral (aggressive behavior), physiological (sleeping time, diurnal activity), pharmacological (tissue distribution of drugs), and biochemical (amine uptake, protein synthesis) effects at various tissue sites. The interaction of stress with either single or multiple exposure to standardized cigarette smoke or to physiological concentrations of nicotine was studied in a number of experiments.

The concentration of acetylcholine, a neurotransmitter, was decreased by 50% or more following stress (Essman, 1972). The central nervous system was particularly vulnerable to the effects of both acute and chronic stress. Electroconvulsive shock led to increases in 5-hydroxytryptamine concentration (17%) and in its metabolism (increased turnover); this effect was further augmented in chronically stressed mice (See Table 1) (Essman, 1973 (2)).

Table 1

Per Cent Concentration Change in Regional 5-Hydroxytryptamine Concentration by Electroconvulsive Shock in Chronically Stressed (Isolation) and Control Mice

BRAIN REGION	CHRONIC STRESS	NO STRESS
Olfactory Bulbs	42*	12.5
Cerebral Cortex	22*	9.5
Mesencephalon	7	21*
Diencephalon	5	23*
Cerebellum	0	25

*p<.02

A relationship between isolation stress, altered brain 5-HT metabolism, and the regulation of brain microsomal protein synthesis was shown (Essman, 1975 (13)). Increased brain 5-HT concentration led to reduced rates of protein synthesis. Nicotine (0.7mg/kg) led to a 59% increase in the rate of microsomal protein synthesis in the cerebral cortex. Changes in brain acetylcholine (ACh) content in both acute and chronic stress were described in similar studies (Essman, 1971). Nicotine produced a reduction in vesicular (52%) and bound (80%) ACh. In stressed mice nicotine uptake into several regions of the brain was significantly increased; i.e., cerebral cortex (96%), hippocampus (43%).

Diurnal changes in brain 5-HT concentration were also related to brain protein synthesis; when endogenous levels of brain 5-HT were high (early AM), the rate of protein synthesis was low; when endogenous levels of this amine were low (PM), high rates of regional protein synthesis were observed (Essman, 1975 (13)). (See Table 2).

Table 2

Diurnal Differences in the
Regional Intercorrelation of ECS or Drug-Induced
Changes in 5-Hydroxytryptamine and Microsomal Protein Synthesis

REGION	AM	PM
Cerebral Cortex	-0.14	-0.74*
Diencephalon	+0.40	+0.76
Limbic Area	-0.94*	+0.56
Cerebellum	+0.67	+0.47

*p .01

Male mice that were isolated developed aggressive behavior but female mice did not. The effects of chronic stress (isolation) in male mice accellerated gonadal (197%), but not adrenal testosterone synthesis. Stressed female mice, although showing a 135% increase in adrenal lipid synthesis, did not show an increased rate of testosterone synthesis (Essman, et al., 1973 (1)).

During the past year, these basic studies have found application in the investigation of stress determinants in mice exposed to tobacco smoke. Various animal holders have been studied to determine their stress effects. The adrenal response to stress has been shown to occur (by elevation of plasma corticosterone (PC) level) by immobilizing mice for intervals as short as 8 minutes. Interestingly, maximal stress, as suggested by PC levels of approximately 180% above baseline (.11 to .15 µg/ml) occurred by 20 minutes of smoking machine confinement, with no subsequent further increase up to one hour (Essman, et al, 1975 (15)). Tobacco smoke introduced into the machine caused no additional elevation of plasma corticosterone content, suggesting that it did not augment the stress response initiated by immobilization.

It was further of interest to observe that adaptation (successive reduction of PC level to within baseline range) would occur with continued exposure to the smoking machine. Adaptation, as measured by decreased plasma corticosterone levels occurred following three weeks of daily exposure to cigarette smoke (8 minutes) for mice immobilized in a smoking maching. Once more, tobacco smoke had no additional effect upon PC levels.

Another measure of stressor effect was cardiac norepinephrine uptake; this was reduced with restraint stress, consisting of 8 minutes of immobilization (66%). Adaptation to stress (gradually increased rates of cardiac NE uptake to

within baseline limits) was also observed after four weeks of daily exposure. No additive or potentiated effects occurred with tobacco smoke, except after 21 days or more of repetitive exposure; at this point cigarette smoke exposure interfered with the stress-induced reduction in NE uptake.

It was possible, through the use of cell fractionation methods, to isolate presynaptic nerve endings (synaptosomes) and their constituent synaptic vesicles. The uptake of 5-HT differed for synaptic vesicles from different mouse strains, C3H showing the lowest uptake and DBA showing the highest. Chronic stress (isolation) reduced vesicular 5-HT uptake for mice of the DBA and C57 strains, but did not alter uptake by vesicles from the C3H strain. Tobacco smoke reversed the effects of stress in C57 strain mice; i.e., smoke exposed C57 strain mice did not show a stress-induced reduction in 5-HT by synaptic vesicles.

Regional (cerebral cortex, diencephalon, cerebellum) uptake of amines (5-HT, norepinephrine, and dopamine) was increased in synaptosomes following in vivo amine depletion; the phenomenon of denervation-supersensitivity (Cannon & Rosenbleuth, 1929) was illustrated in these experiments. This principle holds that a reduction of neural innervation results in a reduction in threshold to activation and increased activity in such cells or tissue. Increased amine uptake by intact nerve endings after a reduction in their number is support for increased sensitivity following reduced innervation.

The question of how basic differences in cellular response to nicotine occurred at physiological concentrations was raised. To deal with this issue, a newly developed tissue fractionation method was used to separate neurons and glia from different brain regions.

In several in vitro studies, it was possible to show that physiological concentrations of nicotine (2.5×10^{-7}M) altered amine uptake by isolated nerve cells and their adjacent glia. Specifically, nicotine increased 5-HT (124%) and dopamine (31%) uptake in diencephalic neurons; in glia from the basal ganglia (30%) and diencephalon (29%) showed increased NE uptake. Decreased dopamine uptake occurred in cortical (76%) and cerebellar (24%) glia; there were no changes in cells of the basal ganglia.

The significance of these findings lies in the regional and cellular specificity with which physiological levels of nicotine act. With amine uptake as an index of decreased receptor availability, one could postulate different regional and cellular sites of excitability change. Cortical and cerebellar excitation may be inferred from the present findings. The absence of any uptake change for cells from the basal ganglia have particular interest since another measure of cellular stimulation is increased protein synthesis -- the basal ganglia represents one region of the brain where the constituent cells show changes in protein synthesis from nicotine.

Protein synthesis was stimulated by physiological concentrations of nicotine, specifically in neurons (59%) and glia (24%) from the basal ganglia; in other brain regions, there was no difference at this observed concentration of nicotine (10^{-7}M). These findings hold some potential clinical significance for Parkinson's disease. Decreased basal ganglia dopamine levels in this disorder have been associated with reduced protein synthesis. It is possible that nicotine could reverse this effect. This relationship bears further investigation.

Tobacco smoke exposure (8 min.) elevated pulmonary 5-HT concentration for certain strains of mice (C3H, DBA, CF1); pulmonary 5-HT content was not altered by tobacco smoke in mice of the C57 strain. These data have been summarized in Table 3.

Table 3

Effect of Tobacco Smoke Exposure
Upon 5-Hydroxytryptamine Content of the Pulmonary
Epithelium of Male Mice of Several Inbred Strains

CONDITION	STRAIN		
	CF-1S	DBA-2	C57/BL6J
Tobacco Smoke	0.22* (0.01)	0.18 (0.01)	0.05 (0.01)
Control	0.18 (0.01)	0.11 (0.02)	0.04 (0.01)

*p <.01

Pulmonary alveolar macrophage yeild was decreased by 32% after 8 minutes of cigarette exposure. Macrophages were shown to bind 5-HT (5.78×10^{-12}Moles); such binding was increased (14.59×10^{-12}Moles) by the gas phase of cigarette smoke, suggesting that the gas phase, in the absence of nicotine increased the number of binding sites on the macrophage. Some strains, such as DBA, yielded macrophages with significantly greater number of binding sites (1.59×10^{-14} Moles), whereas other strains, e.g. C3H, yielded macrophages with an appreciably fewer number of binding sites (0.05×10^{-14}Moles). Tobacco smoke exposure significantly ($p<.01$) decreased 5-HT binding by macrophages (79%) in DBA strain mice, but in no other strain. Stress (isolation) decreased binding in C57 (62%) and DBA strains, but not in C3H mice. Tobacco smoke exposure blocked stress-induced reduction in macrophage binding of 5-HT in C57 mice. These studies suggest strain specificity for (1) macrophage yield, (2) macrophage yield following acute exposure to cigarette smoke, (3) macrophage binding of 5-HT, (4) stress-induced alteration in macriphage binding, and (5) interaction of stress with tobacco smoke in

determining macrophage binding. This appears to be the first report of chronic stress affecting macrophage binding capacity. The ability of tobacco smoke to alter this effect, at least in one strain of mouse, indicates a need for further investigation.